Nuclease

Week 5

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 Plating of cells containing pSB1C3-Nuc from iGEM HQ

Responsible

Oscar He and Ellinor Lindholm

Experimental Setup

Three cell samples were used:
1 iGEM Headquarters
2 Glycerol stock 1 (made from sample from iGEM HQ)
3 Glycerol stock 2 (made from sample from iGEM HQ)

Results and Conclusions

The plating was successful as colonies could be seen.

2 Extraction of pSB1C3-Nuc

Responsible

Ellinor Lindholm and Oscar He

Protocols used

Plasmid Extraction

Modifications and comments to protocols

Plasmid Extraction - Step 1: centrifugation at 13,000 rpm ($\sim$ 17,000 x g)
Plasmid Extraction - Step 10: DNA elution using Buffer EB

Experimental Setup

Table 1: Volumes of the liquid cultures used for plasmid extraction. GS - Glycerol stock, HQ - Headquarters.

Sample	Volume [μl]
Nuc 1.1 GS	4.5
Nuc 1.2 GS	4.5
Nuc 2.1 GS	4.5
Nuc 2.2 GS	4.5
Nuc 1 HQ	4.5
Nuc 2 HQ	4.5

Results and Conclusions

Table 2: Concentrations of extracted plasmid DNA measured using NanoDrop at 280 nm.

Sample	Concentration [ng/μl]
Nuc 1.1 GS	51.8
Nuc 1.2 GS	40.7
Nuc 2.1 GS	56.3
Nuc 2.2 GS	51.9
Nuc 1 HQ	44.8
Nuc 2 HQ	43.8

3 Digestion of pSB1C3-Nuc and pSB1K3-T7-EB (to isolate Backbone) with gel purification and PCR analysis

Responsible

Oscar He, Ellinor Lindholm and Maren Maanja

Protocols used

3A Assembly
PCR Amplification (Q5)
Gel purification
Gel electrophoresis

Modifications and comments to protocols

3A Assembly:	Amount of DNA per reaction:	100 ng
	Number of double digestion duplicates:	3
	Number of single digestion duplicates:	1
	Volume of Enzyme Master Mix (MM) for each double digestion (dd) reaction:	4 μl
	Volume of Enzyme Master Mix (MM) for each single digestion (sd) reaction:	2 μl
	Total Volume of each digestion reaction:	8 μl
PCR Amplification (Q5):		1 μl DNA sample used in PCR mix.
PCR Amplification (Q5) primers:		VR (reverse) VF2 (forward)

Experimental Setup

Table 3: Concentration of samples used for digestion.

Sample	Concentration [ng/μl]
Nuc 2.1 GS	56.3
EB 5.1 (pSB1K3-T7-EB)	194.3

Table 4: Volumes for each component in the enzyme Master Mixes.

Component	MM EcoRI [μl]	MM SpeI [μl]	MM XbaI [μl]	MM PstI [μl]
10x CutSmart Buffer	0.8	-	0.8	1.6
ddH_2O	6.8	-	6.8	13.6
Enzyme	0.4	-	0.4	0.8

Table 5: Volumes of each component used for the digestion mixes.

Component	pSB1C3-Nuc (sd) [μl]	pSB1C3-Nuc (sd) [μl]	pSB1C3-Nuc (dd) [μl]	pSB1K3-T7-EB (sd) [μl]	pSB1K3-T7-EB (sd) [μl]	pSB1K3-T7-EB (dd) [μl]
DNA Sample	1.78	1.78	1.78	0.51	0.51	0.51
MM EcoRI	-	-	-	2	-	2
MM XbaI	2	-	2	-	-	-
MM PstI	.	2	2	-	2	2
10x CutSmart Buffer	0.6	0.6	0.4	0.6	0.6	0.4
ddH2O	3.62	3.62	1.82	4.89	4.89	3.09

Table 6: Weights of the cut out gel bits and volumes of Buffer QG and Iso-propanol used for the gel purification.

Sample	Weights of gel cut out [g]	Buffer QG [μl]	Iso-propanol [μl]
pSB1K3-T7-EB 1.3 dd	0.1767	530.1	176.7
pSB1K3-T7-EB 1.4 dd	0.1436	430.8	143.6
pSB1K3-T7-EB 1.5 dd	0.1605	481.5	160.5
pSB1K3-T7-EB 2.3 dd	0.1743	522.9	174.3
pSB1K3-T7-EB 2.4 dd	0.1643	492.9	164.3
pSB1K3-T7-EB 2.5 dd	0.197	591	197

Sample Calculation

Volume of enzyme stock solution in each enzyme Master Mix

$$Volume\ enzyme = \frac{Amount\ of\ DNA\ per\ reaction}{1000 \cdot (Number\ of\ sd + Number\ of\ dd)} \tag{1}$$

Volume DNA sample in each digestion reaction

$$Volume\ DNA\ sample = \frac{Amount\ DNA\ per\ reaction}{Concentration\ of\ DNA\ sample} \tag{2}$$

Results and Conclusions

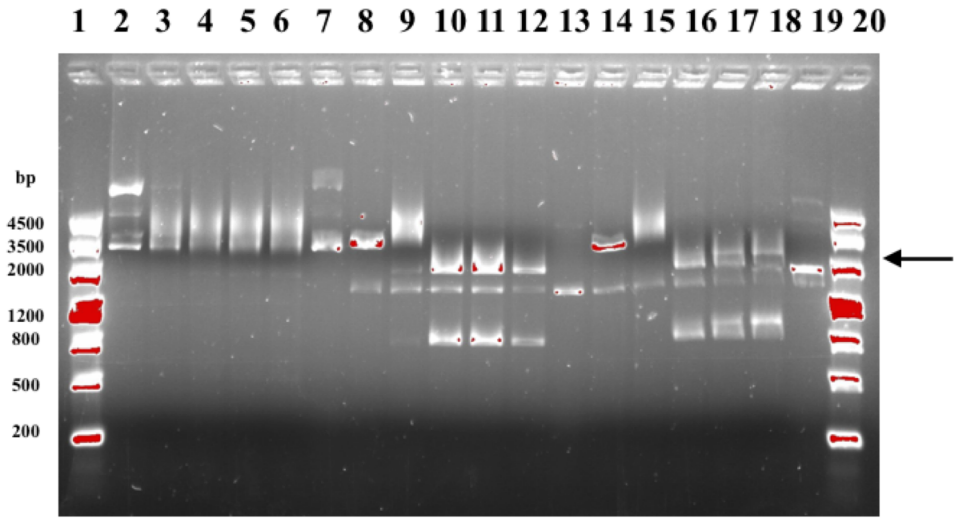

Figure 1: Result of the gel electrophoresis with 1 % agarose at 100 V.

Table 7: The expected size(s) and actual size(s) of the gel electrophoresis in Figure 1.

Well #	Sample	Expected size [bp]	Actual size [bp]
1	DNA Ladder	-	-
2	Nuc 2.1.1 GS sd XbaI	2631	3500 4000
3	Nuc 2.1.2 GS sd PstI	2631	3500-4500
4	Nuc 2.1.1 GS dd	561	3500-4500
5	Nuc 2.1.2 GS dd	561	3500-4500
6	Nuc 2.1.3 GS dd	561	3500-4500
7	Negative control	-	-
8	pSB1K3-T7-EB 1.1 sd EcoRI	3092	3600 900
9	pSB1K3-T7-EB 1.2 sd PstI	3092	3600 2200 900
10	pSB1K3-T7-EB 1.3 dd	2204 888	2200 1700 900
11	pSB1K3-T7-EB 1.4 dd	2204 888	2200 1700 900
12	pSB1K3-T7-EB 1.5 dd	2204 888	2200 1700 900
13	Negative control	-	-
14	pSB1K3-T7-EB 2.1 sd EcoRI	3092	3600 900
15	pSB1K3-T7-EB 2.2 sd PstI	3092	3600 900
16	pSB1K3-T7-EB 2.3 dd	2204 888	2200 1700 900
17	pSB1K3-T7-EB 2.4 dd	2204 888	2200 1700 900
18	pSB1K3-T7-EB 2.5 dd	2204 888	2200 1700 900
19	Negative control	-	-
20	DNA Ladder	-	-

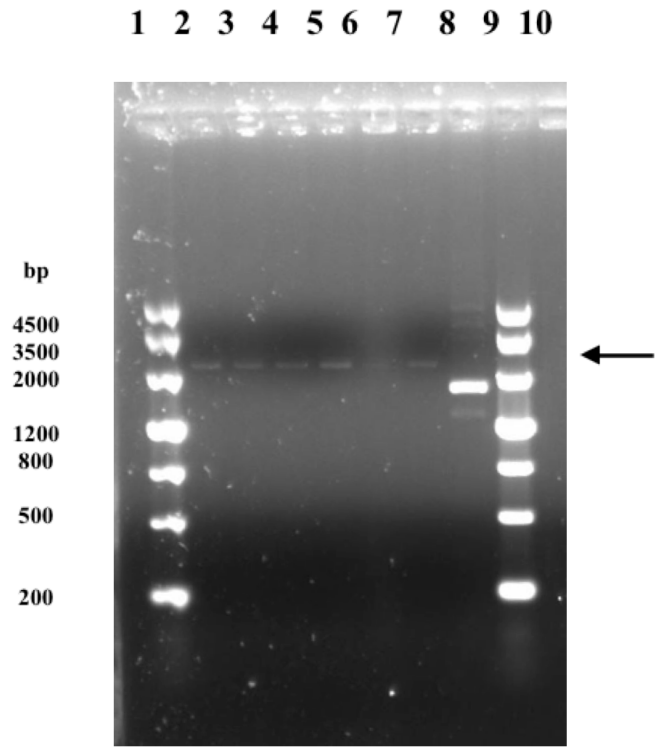

Figure 2: Result of the gel electrophoresis with 1 % agarose at 100 V. (1) DNA Ladder (2) pSB1K3-T7-EB 1.1 dd (3) pSB1K3-T7-EB 1.2 dd (4) pSB1K3-T7-EB 1.3 dd (5) pSB1K3-T7-EB 2.1 dd (6) pSB1K3-T7-EB 2.2 dd (7) pSB1K3-T7-EB 2.3 dd (8) Negative control (9) DNA Ladder.

Table 8: Concentrations of the gel purified samples and after pooling 1.2-2.3.

Sample	Concentration [ng/μl]
pSB1K3-T7-EB 1.1 dd	3.1
pSB1K3-T7-EB 1.2 dd	6.1
pSB1K3-T7-EB 1.3 dd	2
pSB1K3-T7-EB 2.1 dd	3.2
pSB1K3-T7-EB 2.2 dd	2.4
pSB1K3-T7-EB 2.3 dd	6.0
pSB1K3-T7-EB pooled	4.9

Discussion and Troubleshooting

Since the sizes of the bands observed in wells 10, 11, 12 and 16, 17 and 18 (see Figure 1) correspond to the sizes expected of digested backbone, it could be assumed that the digestion was successful. Therefore these bands were extracted from the gel to obtain more backbone used for futuer cloning experiments. These corresponds to a size of the wanted 2200 bp.